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Roles of Fe-S clusters: From cofactor synthesis to iron homeostasis to protein synthesis

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Abstract

Fe-S cluster assembly is an essential process for all cells. Impairment of Fe-S cluster assembly creates diseases in diverse and surprising ways. In one scenario, the loss of function of lipoic acid synthase, an enzyme with Fe-S cluster cofactor in mitochondria, impairs activity of various lipoamide-dependent enzymes with drastic consequences for metabolism. In a second scenario, the heme biosynthetic pathway in red cell precursors is specifically targeted, and iron homeostasis is perturbed, but lipoic acid is unaffected. In a third scenario, tRNA modifications arising from action of the cysteine desulfurase and/or Fe-S cluster proteins are lost, which may lead to impaired protein synthesis. This can then result in cancer, neurologic dysfunction or type 2 diabetes.

Iron-sulfur (Fe-S) cluster assembly

Fe-S clusters are essential cofactors of proteins involved in numerous cellular processes. In eukaryotic cells, Fe-S proteins perform critical functions both inside and outside mitochondria, and their assembly is mediated by multi-subunit machineries termed the ISC (Iron Sulfur Cluster) and CIA (Cytoplasmic Iron-Sulfur Protein Assembly) [for recent reviews [1–3]. The ISC is found primarily in mitochondria in yeast, although in other eukaryotes including humans, ISC components are also found in the cytoplasm and nucleus [2]. The core ISC assembly complex contains the NFS1 cysteine desulfurase, an accessory protein ISD11, frataxin, and the scaffold protein ISCU. NFS1 binds the substrate cysteine and forms a persulfide on its active site. During this process, the enzyme likely undergoes at least two conformational changes for optimum activity. One change is mediated by frataxin interaction that exposes the “buried” substrate-binding sites and promotes substrate binding. A second change is mediated by ISD11 interaction that brings the bound substrate cysteine and the active site cysteine in proximity for persulfide formation [4]. Subsequently, the persulfide sulfur is transferred from NFS1 to ISCU, where it combines with iron to form [2Fe-2S] cluster intermediates. The NADPH-dependent ferredoxin reductase - ferredoxin

redox couple supplies reducing equivalents that are needed for formation of the intermediate. The cluster intermediates are then transferred to apoproteins by the action of HSPA9-HSC20 chaperones. The transfer process also may involve a glutathione-bound intermediate on a monothiol glutaredoxin, GLRX5. Specific subsets of Fe-S cluster proteins are targeted by GLRX5, and allele effects have been noted on target selection [5]. A number of other proteins, including IBA57, NFU1 and BOLA3, are involved in modification of the [2Fe-2S] cluster intermediate into a [4Fe-4S] cluster intermediate and/or in targeting the preformed clusters to specific recipients. The mitochondrial ISC machinery must interface with the CIA machinery in the cytoplasm and nucleus. Interestingly, the CIA machinery bears some similarities to the mitochondrial ISC machinery. For example, scaffold and chaperone components (CFD1/NBP35) have been identified. Likewise, the NADPH-dependent NDOR1 and CIAPIN1 reductase complex is also involved in cytosolic Fe-S cluster synthesis [3]. This article focuses on the roles of Fe-S proteins in three important mitochondrial/cellular functions – biosynthesis of lipoamide that serves as a cofactor for several metabolic enzymes in mitochondria, maintenance of iron homeostasis in mitochondria, and modifications of mitochondrial and cytoplasmic transfer RNAs (tRNAs) required for efficient protein synthesis.

A. Lipoamide cofactor and its biosynthesis

The lipoamide cofactor consists of lipoic acid (a thiol derivative of octanoic acid) bound by an amide linkage to the epsilon amino group of a conserved lysine in a distinct lipoylation domain of some mitochondrial proteins. The cofactor can cycle between a reduced state in which the sulfhydryls are exposed and an oxidized state in which the thiols form a five member ring available for substrate binding. When bound to substrate, the lipoamide adduct acts as a 14Å swinging arm, enabling direct transfer of substrates between enzyme subunits [6]. The lipoamide-requiring enzymes include pyruvate dehydrogenase (PDH), α–ketoglutarate dehydrogenase (α–KGDH), 2-oxoadipate dehydrogenase (2-OADH), branched chain ketoacid dehydrogenase (BCKDH), and glycine cleavage system (GCS) [7] and Fig. 1.

The biosynthesis of lipoic acid enzymes takes place in mitochondria in several steps. The precursor octanoic acid bound by the acyl carrier protein is delivered to recipient proteins and covalently attached by the lipoyltransferase 2 (LIPT2) enzyme to lysine residues on special lipoyl domains. The bound octanoic acid is then modified via the action of a unique mitochondrial Fe-S cluster enzyme, lipoic acid synthase or LIAS [7]. This enzyme carries two critical [4Fe-4S] clusters, an activating cluster and an auxiliary cluster [8]. The proposed mechanism involves interaction of the enzyme with the substrate, the lipoyl carrier protein with bound octanoic acid. The principal cluster mediates activation of adenosyl methionine to a free radical form able to sequentially abstract hydrogens from C6 and then C8 of the substrate. The auxiliary cluster is utilized as a sulfur donor, contributing two equivalents of S^{2-} and “wasting” four equivalents of iron. The auxiliary [4Fe-4S] cluster must therefore be regenerated to process another molecule of substrate [6]. Thus even mild decrease in the activity or flux of the Fe-S cluster assembly system will target LIAS specifically, leading to LIAS deficiency and lipoamide deficiency with drastic metabolic consequences (see below and Fig. 1).

Multiple mitochondrial dysfunction syndrome

A number of devastating inherited metabolic diseases have recently been described that are characterized by lipoamide deficiency [7, 9]. These diseases are referred to as multiple mitochondrial dysfunction syndrome. The disease manifestations have considerable overlap, but interestingly, a number of genes/proteins involved in general Fe-S cluster assembly have been implicated, including IBA57 [10], NFU1 [11], BOLA3 [5] and GLRX5 [5]. Primary deficiencies of LIAS also share many of these features, such as non-ketotic hyperglycemia, seizures, brain damage, elevated plasma glycine and defective mitochondrial energy metabolism [5]. The constellation of symptoms and findings derive from accumulation of toxic oxo-acids on the one hand or energy deficiency on the other hand [7]. Mitochondria are also globally defective. Toxic oxo-acids, especially glycine, which must be handled by the glycine cleavage enzyme but also ketoacids and lactate, accumulate in the plasma and central nervous system. Here they may act as neurotoxins producing various neurologic symptoms such as intractable seizures, encephalopathy, hypotonia, brain malformations, and developmental regression. Non-ketotic hyperglycemia may result from pyruvate dehydrogenase deficiency. On the other hand, lack of energy as may result from decreased acetyl-CoA production and decreased metabolic flux through the mitochondrial electron transport chain may produce a broad spectrum of disease symptoms that alter functions of skeletal muscle (myopathy), heart (cardiomyopathy), and lungs (respiratory insufficiency) [7, 9].

B. Mitochondrial iron homeostasis - Defects in Fe-S cluster biogenesis causing sideroblastic anemia

Sideroblastic anemia is a hematologic syndrome characterized by iron accumulation in mitochondria of red cell precursors in the bone marrow leading to decreased viability of these precursors and anemia [12, 13]. Sideroblastic anemia reflects a problem with iron utilization for heme synthesis. In some cases, its pathogenesis has been linked to deficiency of Fe-S cluster synthesis. The manner in which Fe-S cluster deficiency causes mitochondrial iron accumulation is not entirely clear [14]. However, critical Fe-S cluster proteins have been implicated in mediating or regulating heme synthesis. For example, ferrochelatase, the enzyme that inserts iron into porphryin in mitochondria is a [2Fe-2S] cluster protein, and the cluster is necessary for the activity and stability of the protein, although it is not directly involved in the enzymatic activity [15]. The iron regulatory protein 1 (IRP1) in cytoplasm is another Fe-S cluster protein that acts as a regulatory switch for the heme/porphyrin biosynthetic pathway. When it assembles with a [4Fe-4S cluster] it functions as the cytosolic aconitase, but when Fe-S clusters (or iron) is scarce, the apoprotein binds to the 5′ regulatory stem loop in the amino terminal 5′ UTR of aminolevulinic acid synthase mRNA, repressing translation of this key rate-limiting enzyme for porphyrin synthesis [16]. Interference with Fe-S cluster biogenesis then may abrogate heme synthesis by blocking porphyrin synthesis at the first and last steps. Genes implicated in Fe-S cluster assembly and causing sideroblastic anemia include GLRX5 and ABCB7; SF3B1 has been linked to aberrant splicing of ABCB7 mRNA [17].

GLRX5 alleles, one gene and two diseases

An individual carrying inherited compound heterozygous mutations of GLRX5 L148S and K101Q was found to develop sideroblastic anemia [18]. A biochemical analysis of the alleles was performed by expressing them from plasmids in a K562 hematopoietic cell line with CRISPR/Cas9 inactivation of the native GLRX5 [19]. In the cell line, the L148S or coexpressed L148S and K101Q alleles were associated with Fe-S cluster deficient phenotypes: IRP1 was lacking its Fe-S cluster and c-aconitase activity was markedly down. The Fe-S cluster containing protein ferrochelatase was also decreased in amount. The iron-associated phenotypes apparent in these cells included an increase in transferrin receptor levels, increased cellular and mitochondrial iron, and decreased total heme levels [19], consistent with the sideroblastic anemia phenotype. However, not all Fe-S cluster proteins were deficient, and succinate dehydrogenase was normal in spite of the fact that it requires three different Fe-S cluster cofactors – [2Fe-2S], [3Fe-4S] and [4Fe-4S] – for its activity. In addition, lipoic acid dependent enzymes, pyruvate dehydrogenase and α-ketoglutarate dehydrogenase, were entirely normal, suggesting that lipoate formation via the FeS cluster-requiring enzyme LIAS was preserved [19].

A different homozygous mutant allele of GLRX5, K51del, was identified in an individual with an inherited metabolic syndrome. The clinical features included early age of onset at 3 years, non-ketotic hyperglycemia, psychomotor retardation, and glycine accumulation in blood and cerebral spinal fluid [5]. The latter probably resulted from deficient glycine cleavage enzyme activity, a lipoic acid-dependent function. Other lipoic acid-dependent enzymes, PDH and α–KGDH, were also very deficient, probably as a result of loss of activity of the Fe-S cluster enzyme, LIAS [7]. However, in the engineered K562 cells expressing only the K51del allele, cytoplasmic and mitochondrial aconitases and ferrochelatase were unaffected [19]. Accordingly in the patient with the K51del allele and inherited metabolic syndrome, the blood picture was entirely normal and sideroblastic changes were not seen [5].

ABCB7 involvement in inherited and acquired sideroblastic anemia

ABCB7 is an ATP-dependent mitochondrial transporter implicated in Fe-S cluster assembly [3]. The orientation of the transporter in the mitochondrial inner membrane with its ATP and substrate-binding sites in the mitochondrial interior suggests that it functions as an exporter [20]. The precise substrate is still unidentified, but likely candidates include sulfur species such as polysulfides or glutathione derivatives [21]. Biochemical studies suggest that NFS1/ISD11, the mitochondrial cysteine desulfurase, is required to generate the substrate from cysteine. Subsequent transfer of the substrate into the cytoplasm via ABCB7 may be necessary for Fe-S cluster assembly on cytoplasmic Fe-S cluster substrates such as IRP1. Inherited mutations in ABCB7 have been linked to XLSA-A, X-linked sideroblastic anemia with cerebellar ataxia [22]. The male predominance of the disease is consistent with the X-chromosomal location of the ABCB7 gene. The neurologic features are more striking than the hematologic features, and the cerebellar atrophy and motor impairment present early on, whereas the sideroblastic anemia presents later and is relatively mild [23]. The mechanism by which the ABCB7 deficit causes iron homeostatic changes leading to sideroblastic

anemia probably involves defective formation of a regulatory Fe-S cluster outside mitochondria but the identity of this putative Fe-S cluster target is unknown.

SF3B1 involvement in sideroblastic anemia and myelodysplasia

SF3B1 encodes an essential component of the spliceosome [24]. The protein is involved in RNA splicing, mediating recognition of the branch point sequence and selection of the 3′ splice site. SF3B1 hotspot mutations are neomorphic and induce aberrant 3′ splice site selection [17]. Thus, it is the K700E allele of SF3B1, and not loss-of-function mutations, that have been strongly associated with the development of sideroblastic anemia [25]. Apparently this allele leads to altered branch point selection and altered splicing of a large number of target genes, including heme biosynthetic enzymes, cell cycle enzymes and DNA repair enzymes. Most interestingly, SF3B1 with the K700E allele results in the aberrant splicing of the ABCB7 mRNA, introducing a frame shift at the 3′ splice site. The frame shift in turn results in nonsense mediated decay of the mRNA and downregulation of expression [17]. The effects of ABCB7 downregulation on Fe-S cluster assembly outside mitochondria are likely to be similar to what occurs in the inherited XLSA-A syndrome. Various features of sideroblastic anemia result (e.g. heme deficiency, iron misregulation, mitochondrial iron accumulation and ineffective erythropoiesis), although the causal link with these outcomes has not yet been directly demonstrated.

In the SF3B1 associated sideroblastic anemia, as opposed to the inherited form, the abnormalities are confined to hematopoietic cells [25]. Sideroblastic anemia is considered to be a variety of myelodysplasia, an acquired bone marrow disease caused by somatic genetic changes in a clone or clones of hematopoietic stem cells[26]. These clonally derived cells have a survival advantage in the bone marrow, but as they mature into precursors for the different blood lineages, they die prematurely (ineffective hematopoiesis) (Fig. 2). In some cases, SF3B1 mutations are associated with additional genetic changes that give rise to other cytopenias or myeloproliferative features (12).

C. Fe-S clusters and tRNA modifications

tRNAs are fundamental components of the protein-synthesizing machinery. Many tRNAs contain conserved nucleotide modifications, and these modifications are often required for accurate and efficient recognition of codons in mRNA, maintenance of proper reading frame, and/or tRNA structural stability. It is therefore not surprising that defects in tRNA modifications are associated with numerous diseases including cancer, neurological disorders, and type 2 diabetes [27–29].

Many tRNAs have modifications at position 34 corresponding to the wobble position of the anticodon, and at position 37 that is adjacent to the 3′-position of the anticodon. For example, cytoplasmic tRNAs are usually modified by the covalent addition of methoxycarbonylmethyl (mcm) or carbamoylmethyl (ncm) to the 5′ carbon of the wobble base uridine (U34). A subset of these tRNAs - $tRNA_{Lys}$ (UUU), $tRNA_{Glu}$ (UUC), and $tRNA_{Gln}$ (UUG) – the U34 base is further decorated with a thio group, replacing oxygen at 2′ position [30]. In *Saccharomyces cerevisiae*, loss of thiolation does not eliminate mcm^5

modification, and cells lacking either mcm^5 or s^2 are viable. However, simultaneous loss of mcm^5 and s^2 is lethal [31]. Other derivatives such as 5-carboxymethylaminomethyl-2-thiouridine ($cmnm^5s^2U$) and 5-taurinomethyl-2-thiouridine (τm^5s^2U) are found in yeast and mammalian mitochondrial tRNAs, respectively [30].

The modifications at position 37 often form hyper-modified nucleosides such as N^6-isopentenyladenosine (i^6A) and its 2-methylthio derivative (ms^2i^6A), N^6-threonyl-carbamoyladenosine (t^6A) and its 2-methylthio derivative (ms^2t^6A), and wybutosine (yW). Interestingly, several enzymes involved in these modifications contain Fe-S clusters (see below and Table 1). Furthermore, like in the case for Fe-S clusters and lipoic acid, the source of sulfur for tRNA thiolation is also the persulfide sulfur formed on the NFS1 cysteine desulfurase. For tRNA thiolation, methylthiotransferases are responsible for the s^2 modification. However, unlike in the case for lipoic acid biosynthesis, the [4Fe-4S] clusters of methylthiotransferases do not appear to be sacrificed as sulfur donors [32].

TYW1 (tRNA-yW synthesizing protein 1)

TYW1 is found in cytoplasm; it contains two [4Fe-4S] clusters and is involved in wybutosine (yW) biosynthesis. yW biosynthesis occurs by a series of post-transcriptional modifications of the genetically encoded guanine base at position 37 of eukaryotic $tRNA^{Phe}$. The initial steps involve methylation of N1 at G37 to form N-methylguanosine (m^1G), which is then converted to 4-demethylwyosine (imG-14) by TYW1. Further modifications of imG-14 to yW require several other enzymes [33, 34] (Table 1).

yW is a tricyclic nucleoside that contributes to translational fidelity. It stabilizes the codon anticodon pairing through an increased base-stacking interaction that helps in maintaining the reading frame [35]. Lack of yW may therefore cause increased frame-shifting during translation [36]. For example, yW is absent in $tRNA^{Phe}$ in cells infected with human immunodeficiency virus (HIV). The lack of yW in these cells causes a greatly enhanced -1 frame-shifting, allowing the HIV virus to produce the reverse transcriptase that is essential for virus replication and activity [37]. The lack of yW in $tRNA^{Phe}$ could be specifically due to malfunction of TYW1 in HIV-infected cells, although this remains to be established.

CDKAL1 (Cdk5 regulatory subunit associated protein 1-like 1)

CDKAL1 is a methylthiotransferase that contains two [4Fe-4S] clusters. The enzyme catalyzes the 2-methylthio (ms^2) modification of N^6-threonyl-carbamoyladenosine (t^6A) in tRNA to 2-methylthio-N^6-threonyl-carbamoyladenosine (ms^2t^6A) at position 37 of $tRNA^{Lys}$ (UUU). Lack of this ms^2 modification in $tRNA^{Lys}$ (UUU) leads to abnormal proinsulin synthesis, ultimately causing impaired glucose metabolism and development of type 2 diabetes [38, 39] (Table 1).

CDK5RAP1 (Cdk5 regulatory subunit-associated protein 1)

It is a methylthiotransferase in mammalian mitochondria. It is homologous to CDKAL1, and like CDKAL1, CDK5RAP1 also contains two [4Fe-4S] clusters required for ms^2 group insertion. CDK5RAP1 specifically converts N^6-isopentenyladenosine (i^6A) to 2-methylthio-

N^6-isopentenyladenosine (ms^2i^6A) at A37 of tRNAs for Phe, Ser, Trp, and Tyr in mammalian mitochondria (Table 1). In CDK5RAP1 knockout mice, a deficiency in the ms^2 modification of i^6A resulted in impaired mitochondrial protein synthesis and respiratory defects. Furthermore, the knockout mice exhibited accelerated myopathy and cardiac dysfunction particularly under stressed conditions [40]. Interestingly, the ms^2 modification was also found to be reduced in patients with mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes (MELAS) carrying the A3243G mutation in mitochondrial $tRNA^{Leu}$. However, mt-$tRNA^{Leu}$ does not contain an ms^2 modification. Instead, $tRNA^{Leu}$ carrying the A3243G mutation is deficient in taurine modification and this defect appears to be the primary cause of MELAS [41]. The myopathy in MELAS could be due to combined effects of decoding errors occurring at multiple codons.

ELP3 (Elongator protein 3)

The Elongator complex has been shown to associate with actively transcribing RNA polymerase II. The eukaryotic Elongator complex consists of six Elongator protein subunits (ELP1–ELP6), and the complex is conserved from yeast to humans. Defects in the ELP complex elicit pleiotropic phenotypes involving a variety of cellular processes that include histone acetylation, transcription, and exocytosis. Remarkably, all of these phenotypes appear to originate primarily from the lack of modifications at position 34 of some tRNAs. In fact, all of the six subunits of the ELP complex (together with the Killer toxin-insensitive proteins) are required for formation of mcm^5U, mcm^5s^2U, and ncm^5U at the wobble position [27–29] (Table 1).

Among the components of the Elongator complex, ELP3 is generally considered the catalytic subunit. Only ELP3 has a [4Fe-4S] cluster, and it has two enzymatic domains – a radical S-adenosylmethionine (SAM) near the N-terminus, and a histone acetyltransferase (HAT) at the C-terminus of the protein. Interestingly, both enzymatic activities are necessary for ELP3 functions [27, 42]. In an association study, allelic variants of ELP3 identified in three human populations were found to be associated with amyotrophic lateral sclerosis (ALS) [43]. Likewise, neuronal defects have also been observed in mouse, flies, zebrafish, and *C. elegans* with impaired ELP3 function [27, 29, 44].

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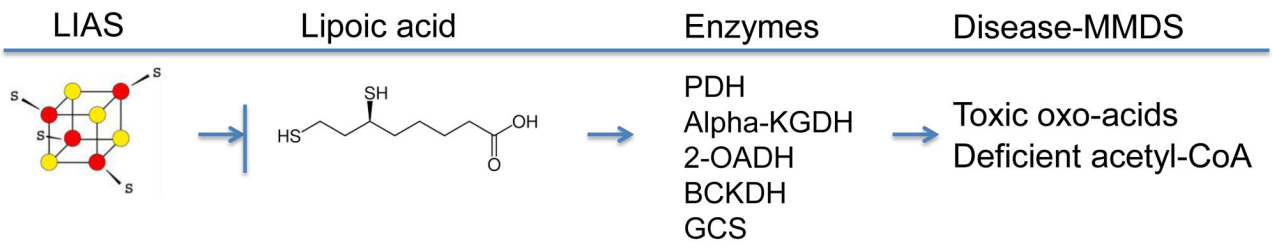

Fig. 1.

Linkage of Fe-S cluster assembly defects and MMDS multiple mitochondrial dysfunction syndrome. Details are included in the text.

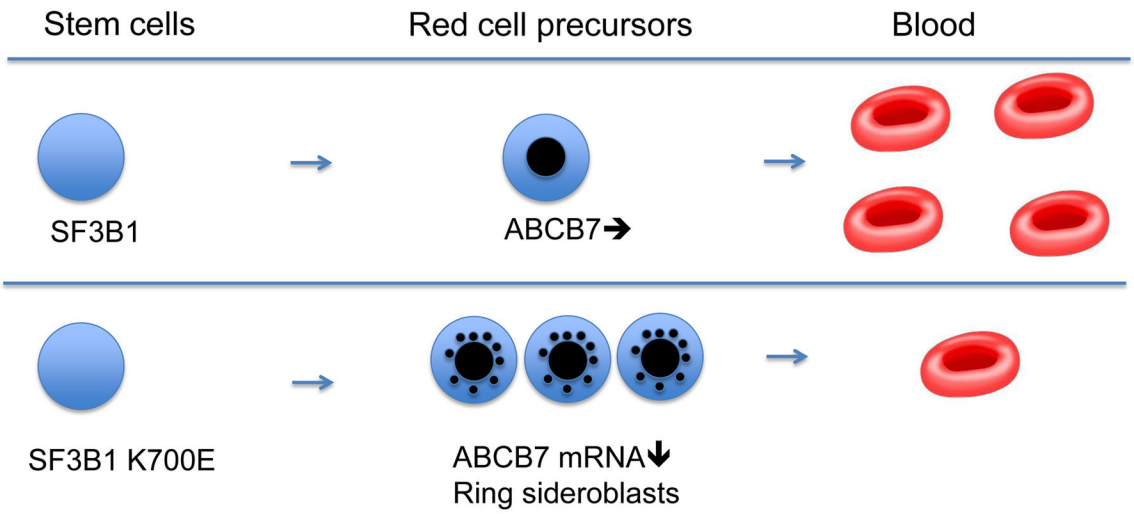

Fig. 2.

Schematic showing possible causation of acquired sideroblastic anemia. Upper panel: normal hematopoiesis. Lower panel: SF3B1 K700E allele in a bone marrow stem cell causes altered splicing of ABCB7, non-sense mediated decay, and ring sideroblast formation in red cell precursors. Red cell precursors expand in the bone marrow but viable circulating red cells are decreased (ineffective erythropoiesis).

Table 1

tRNA modifications mediated by Fe-S cluster enzymes, and defects associated with impaired modifications

Enzyme	[4Fe-4S] clusters	Localization	tRNA	Substrate	Product	Abnormalities associated with impaired tRNA modifications	Refs.
TYW1	Two	Cytoplasm	$tRNA^{Phe}$ (position 37)	m^1G	imG-14	More prone to frame-shifting. Impaired modifications found in HIV-infected cells, Ehrlich ascites tumor, and neuroblastoma cells	32–36
CDKAL1	Two	Associated with ER	$tRNA^{Lys}$ (position 37)	t^6A	ms^2t^6A	Pancreatic islet hypertrophy, impaired blood glucose control, and increased risk of type 2 diabetes in humans	37, 38
CDK5RAP1	Two	Mitochondria	Various (position 37)	i^6A	ms^2i^6A	Defective mitochondrial protein synthesis, respiratory deficiency, and myopathy	39
ELP3	One	Cytoplasm	Various (position 34)	U	cm^5U	Neuronal defects observed in mouse, zebrafish, Drosophila, and *C. elegans*; increased risk of ALS in humans	26–28, 41–43